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Chromosomal reduction in an Okapi pedigree (*Okapia johnstoni*)

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Abstract

The karyotype of a female okapi showing 2n = 44 has been investigated by G-R-C and Ag-NOR banding methods. This animal is the offspring of a captive female and a wild-caught male, which are both heterozygotes showing 2n = 45 with centric fusion between two unequal-sized acrocentric chromosomes. The okapi karyotype was arranged according to the cattle standard karyotype in consideration of the high degree of banding homologies found between the two species. The reduction from 2n = 46 to 2 = 45 and 2n = 44 is the result of a Robertsonian translocation involving cattle equivalent chromosomes 4 and 26. Other autosomal rearrangements, like centric fusions and a tandem translocation, as well as structural changes in the X and the Y chromosomes, are tentatively identified. The location of centromeric heterochromatin and of nucleolus organizer regions in the okapi karyotype are described and discussed in relation to the karyotype in other species of Bovidae.

Introduction

The place of the Giraffidae, including the okapi, in the mammalian systematics is still controversial (Gijzen 1959; Taylor et al. 1969; Romer and Parsons 1986). Before the advent of the banding techniques, the chromosomes of the okapi (*Okapia johnstoni*), one of the two surviving species in the family Giraffidae, were first examined by Ulbrich and Schmitt (1969) showing 2n = 46. Subsequently, Hösli and Lang (1970) studied two other animals with 2n = 45. Later Benirschke et al. (1983) confirmed the fusion between two acrocentric autosomes as a common cytogenetic event referred to as "Robertsonian" without phenotypical changes in animals with reduction from 46 to 45 chromosomes. Using various banding methods, Petit and Meurichy (1986) studied two other animals showing 2n = 46 and 2n = 45 in a female and a male okapi, respectively. We were also able to notice the centric fusion between two unequal-sized acrocentrics in the male heterozygote animal with 2n = 45. We report here the cytogenetic studies of a fertile female animal with 2n = 44 (Studbook No 328) showing two translocated elements as the chromosomal origin of her homozygote status and of her male son with 2n = 45 (Studbook No 403), respectively. The okapi "nombre fondamental" (NF) of 60 is very close to the most common bovid NF of 60, therefore, karyotypes of these animals were performed according to standardized cattle banded karyotype (ISCNDA 1989).

Material and methods

Figure 1 illustrates the pedigree prepared from the International Studbook of the Okapi (Puijenbroeck and Bois 1991). Animals No 257 and No 273 were previously reported on by Petit and Meurichy (1986). The diploid chromosome number of okapi No 283 was established in a North-American zoo. To our knowledge, animals No 311 and No 343, have not yet been karyotyped. Chromosome studies of the remaining animals (Studbook Nos 219, 403) were routinely performed by

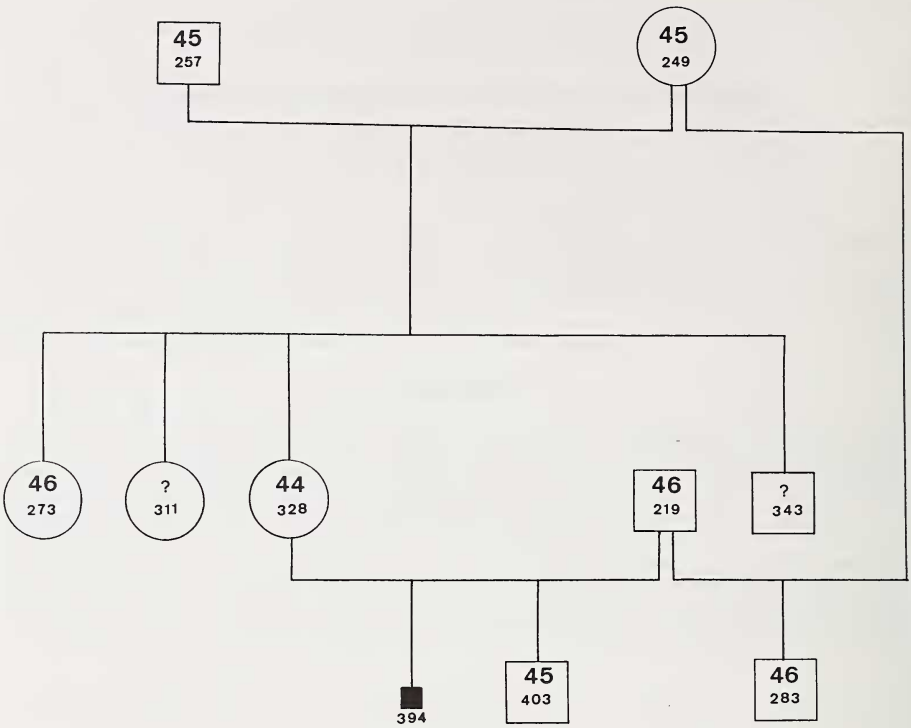

Fig. 1. Pedigree of okapi with studbook numbers in lowercase and chromosome numbers in uppercase. 46 indicates diploid number with absence of t(4;26); 45 and 44 heterozygote and homozygote for t(4;26), respectively indicates male stillborn and ? that chromosome number is unknown

us. The metaphases were observed from cultured skin biopsies, applying to medium and high resolution G and R coloration banding methods (Pai and Thomas 1980; Yunis et al. 1978). C-bands were stained applying to the method of Sumner (1972). Nucleolus Organizer Regions (NORs) have been located using the silver staining technique (Goodpasture and Bloom 1975). The okapi karyotypes were constructed from at least ten well banded metaphase spreads in each case and were prepared according to the standardized cattle banded karyotypes (ISCNDA 1989).

Results

The G-banded karyotypes of two animals demonstrating the reduction from 2n = 45 to 2n = 44 are illustrated in figure 2. In the female with 2n = 44 seven pairs of submetacentric and fourteen pairs of acrocentric chromosomes are numbered according to the cattle nomenclature (ISCNDA 1989). As a consequence of this, the seven biarmed chromosomes are identified as follows: t(27;2;23), t(4;26), t(5;29), t(10;24), t(14;25), t(17;18), t(20;22). The t(27;2;23) is a Robertsonian type 2;23 translocation and 2;27 a tandem translocation. The centromeric region of the chromosome 27, fused at the telomeric region of 2, could not be identified. This t(27;2;23) has been identified previously as t(2;22) by Petit and Meurichy (1992). The translocation event which concerns the chromosomal homozygosity in the 2n = 44 female okapi is identified as t(4;26). The remaining acrocentric okapi chromosomes appear similar in G- and R-banding to the banding patterns of cattle homologues (Fig. 3).

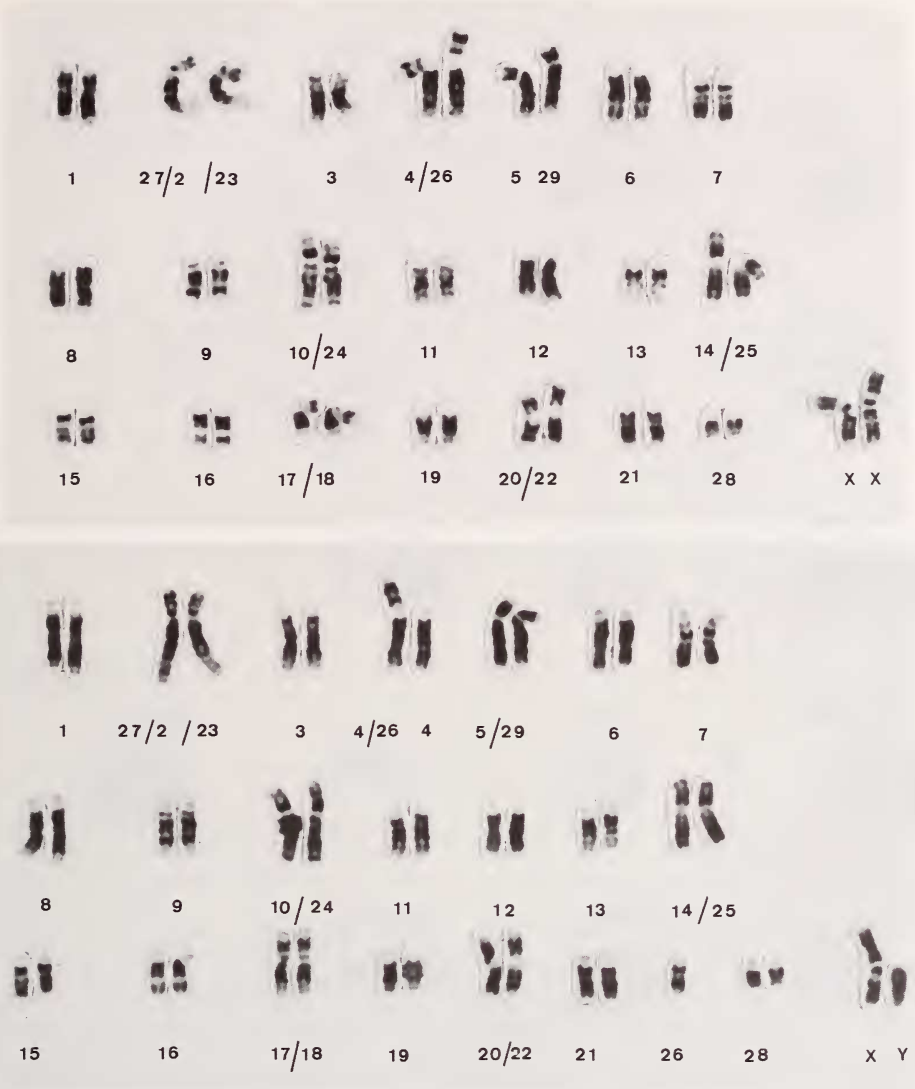

Fig. 2. a: G-bandes karyotype of the 2n = 44 female okapi (Studbook No 328) showing the two t(4;26) elements. b: G-banded karyotype of 2n = 45 male heterozygote (Studbook No 403)

In the male animal with the modal number of 2n = 45 heterozygosity for t(4;26) is demonstrated in figure 2. After using C-banding, small amounts of centromeric heterochromatin (HC) are observed in all the biarmed chromosomes as in t(27;2;23), t(5;29), t(10;24), t(17;18), respectively, whereas in the two remaining translocation elements t(4;26) and t(20;22) much larger HC blocks are demonstrated (Fig. 4). After NOR banding silver dots are observed located in the centromeric region of chromosomes 3, 6, 11, and 28, respectively (Fig. 5).

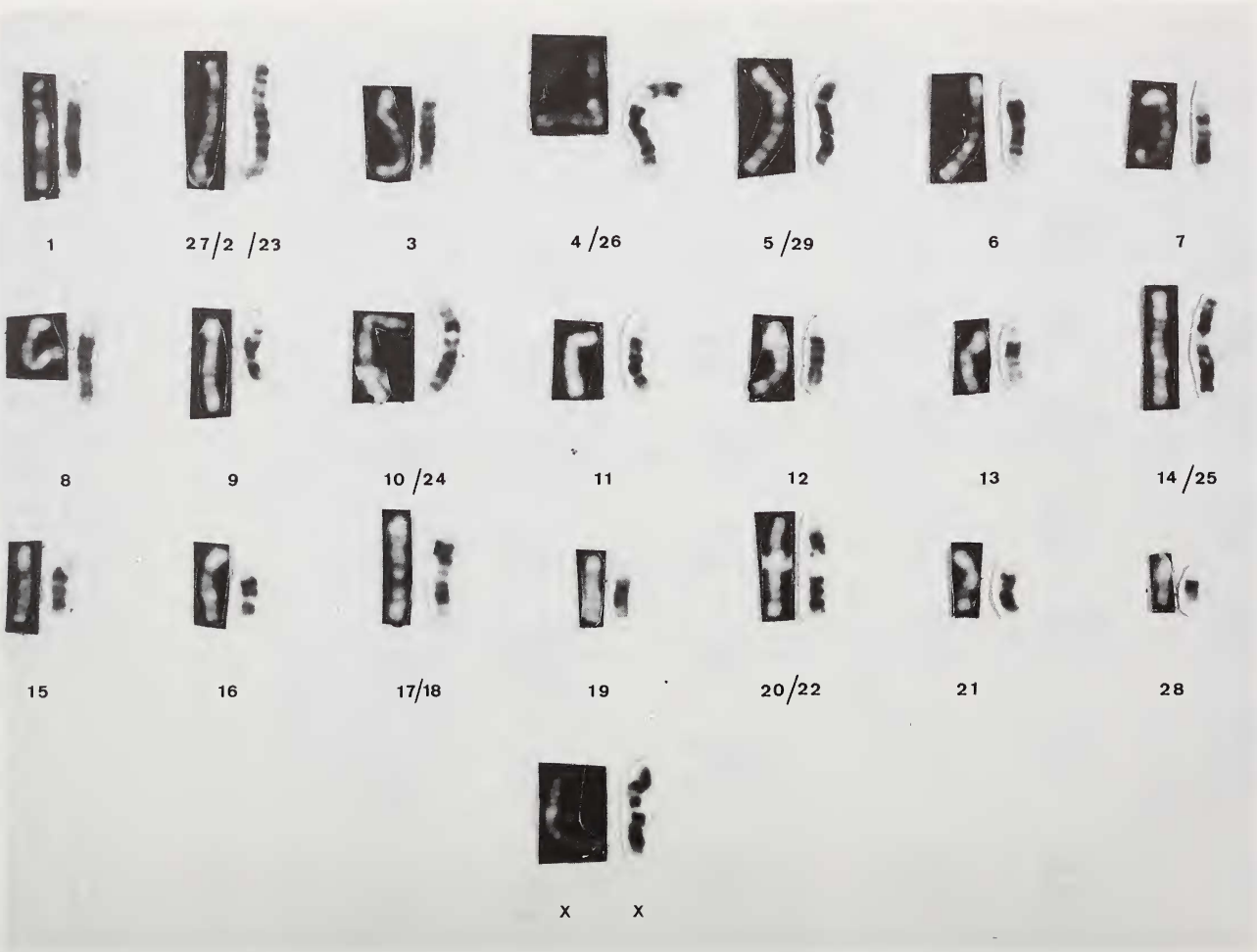

Fig. 3. Combined R-G-banded haploid karyotype of the 2n = 44 animal

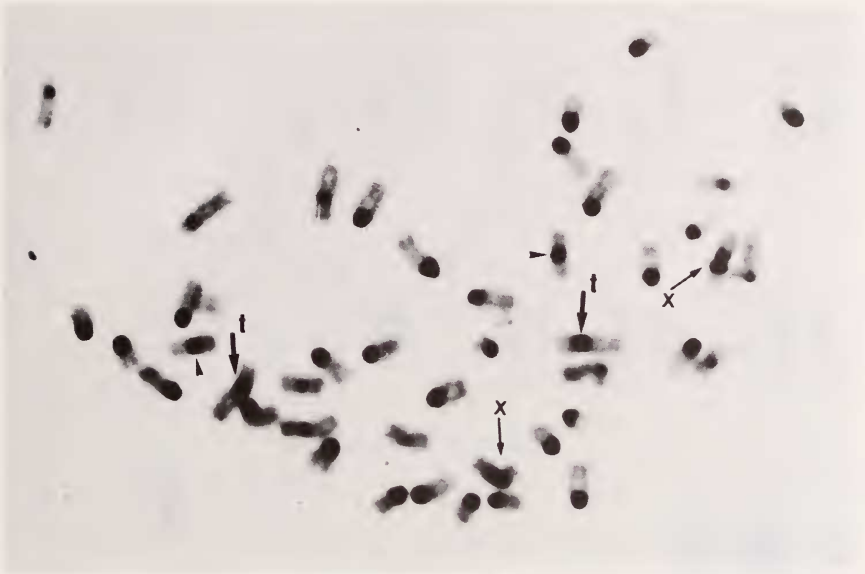

Fig. 4. C-banded metaphase chromosomes of the 2n = 44 animal showing large heterochromatin blocks in two t(4;26) and t(20;22) elements (large arrows: t) and an additional interstitial C-band close to the centromeric C-band in the X chromosomes (small arrows). Note also reduced amounts of HC in the other biarmed chromosomes except for t(4;26) and t(20;22) (arrowheads)

Discussion

From a survey of 21 okapi, Benirschke et al. (1983) reported that 8 animals had 46 chromosomes and 13 had 45 chromosomes. These authors demonstrated that the reduction from 46 to 45 chromosomes was the result of a Robertsonian translocation between two unequal acrocentrics without deleterious effect in the carriers. Recently 43 animals have been karyotyped, among a captive population of 74 animals, showing that 18 animals possess 46 and 25 only 45 chromosomes (Puijenbroeck and Bois 1991). However, the geographical origin of the t(4;26) remains unknown and was most likely imported from the wild as suggested by Benirschke et al. (1983). Thus, the conception of an okapi animal with 2n = 44 resulting from mating of a captive mother (No 249) with a wild-caught father (No 257) would occur at a frequency of 25 % when two 2n = 45 animals are crossed. In this report a 2n = 44 fertile female (No 328) gave birth to a male stillborn (20 kg) with 2n = 45 (No 394) without visceral anomalies at autopsy, and later to a second healthy male (No 403) with 2n = 45. Furthermore, the history of this 2n = 44 female okapi did not reveal either miscarriages or aborted malformed fetuses as a result from chromosomal malsegregation. According to Fosse (1978) neonatal mortality rate in the captive okapi population was high but has been virtually eliminated in the United States in contrast to the European okapi population (Bois et al. 1988).

Cytogenetically, the distribution of HC, as revealed by C-banding, has demonstrated interesting features in the okapi karyotype. At first, the well marked amount of HC in the acrocentric autosomes is conform with observations in cattle (Buckland and Evans 1978b). Secondly, in the pericentromeric regions of the fused elements t(4;26) and t(20;22) large blocks of HC contrast with the small blocks in the 5 other translocated autosomes. Similarly, large blocks of HC on t(15;25) in contrast with a small block on t(1;29), has been

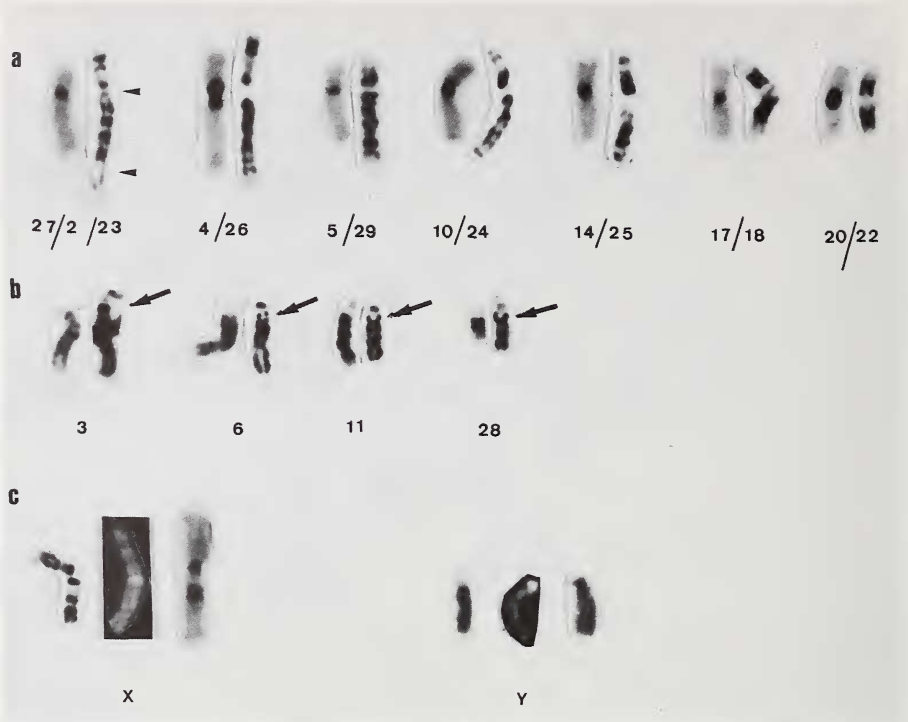

Fig. 5. a: The seven bi-armed okapi chromosomes after C- and G-banding. Arrows indicate the breakpoints in the composite t(27;2;23). b: Acrocentric autosomes 3, 6, 11, and 28 after consecutive G-banding (left) and NOR staining dots (arrows). c: Representative okapi sex chromosomes obtained by G-R-C-banding from left to right

reported in a 59,XX Portuguese Barrosa cow (Iannuzzi et al. 1992). These findings imply that translocated chromosomes, involving fusion of different bovid acrocentric autosomes, may initially contain large blocks of HC which are reduced in size with time. Thus, recent or "new" Robertsonian translocations should progressively loose blocks of HC in the biarmed chromosomes during further evolution.

With regard to the Robertsonian fusions, the presence of the 2;23 translocation in the river buffalo (2n = 50) and in the anoa (2n = 46) was also demonstated but without additional translocated chromosome 27 at the telomeric region of chromosome 2 (Iannuzzi et al. 1990). As a consequence, the resolution of the composite t(27;2;23) characterizes the okapi karyotype. Comparison of the okapi karyotype with a recent cytogenetic survey of 12 bovid species, has indicated considerable monobrachial G-band homologies, but few biarmed chromosome homologies (Gallagher and Womack 1992). Interestingly we have found that the okapi not only shares t(14;25) and t(17;18) with the Roosevelt gazelle (2n = 30) but has the t(20;22) in common with the topi (2n = 36). These findings emphasize that certain biarmed homologies are identical among the translocation events which have arisen independently in three Artiodactyla groups, i.e. the Giraffidae, the Antilopinae and the Alcelaphinae (Buckland and Evans 1978a; Gallagher and Womack 1992). In contrast, no homologous biarmed chromosomes have been observed in a comparative study between the okapi and the giraffe showing 2n = 30 with NF = 58 (Petit and Meurichy 1992). However, the metacentric morphology of the X chromo-

some in these related animals is strikingly different from submetacentric X chromosomes in cattle (Petit and Meurichy 1992; Iannuzzi 1990). The large okapi Y chromosome is similar in size to those of the river buffalo and the anoa (Iannuzzi et al. 1990). From previous studies, heterochromatisation of the okapi long arms is evident after C-banding (Petit and Meurichy 1992). According to Matthews and Reed (1991) the large size of the Y chromosome could be considered as resulting from a class of repeated DNA sequences that is represented in the male bovine genome. This could be related to the phenomenon of acquisition of large amounts of HC material, leading to the morphology of the okapi Y chromosome.

Usually, NORs are observed in telomeric positions in the Bovidae (Mayr et al. 1985; Meo et al. 1991). In contrast, we have identified four acrocentric autosome pairs numbered 3, 6, 11 and 28 demonstrating NORs close to the centromeres. Although pericentromeric location of these NORs have been observed by us in several okapis, it remains unclear why the location of NOR in the okapi is centromeric, while in the Bovidae no other examples have yet been described.

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Zusammenfassung

Chromosomenreduktion in einer Okapi-Familie

Der Karyotyp eines weiblichen Okapi, das 2n = 44 aufwies, wurde mittels der G-R-C und Ag-NOR-Bänderung untersucht. Dieses Tier ist das Resultat einer Paarung zwischen einem aus Tierhaltung stammenden Weibchen und einem in Freiheit aufgewachsenen Männchen, welche beide heterozygot sind und 2n = 45 mit einer zentrischen Fusion zwischen zwei ungleich großen akrozentrischen Chromosomen zeigen. Der Okapi-Karyotyp wurde anhand eines Rinderstandards unter Berücksichtigung des hohen Grades an Bandenhomologie zwischen den beiden Spezies angefertigt. Die Reduktion von 2n = 46 auf 2n = 45 und 2n = 44 ist das Resultat einer Robertson'schen Translokation, die das Rinderäquivalent von Chromosom 4 und 26 einbezieht. Abgesehen von den autosomalen Veränderungen wurden auch Tandemtranslokationen und strukturelle Änderungen bei X- und Y-Chromosomen identifiziert. Die Anordnung von zentromerischen Heterochromatin und Nucleolus organisierenden Regionen im Okapi-Karyotyp werden unter Bezugnahme auf den Karyotyp anderer Arten der Bovidae beschrieben und diskutiert.

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